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- 1) Crompton et al. Journal of General Virology. 1994; 75 (pt 1): 133-139.
- 2) Yang et al. Human Gene Therapy. 1998; 9/13: 1929-1937.
- 3) Douglas et al. Nature Biotechnology. Nov. 1996; 14 (11): 1574-1578.
- 4) Hallenbeck et al. (Advances in Experimental Medicine and Biology. 2000; 465: 37-46.

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Article title	Targetable gene delivery vectors
Article identifier	0065259800135677
Authors	Hallenbeck_P_L Stevenson_S_C
Journal title	Advances in Experimental Medicine and Biology
ISSN	0065-2598
Publisher	Plenum
Year of publication	2000
Volume	465
Issue	2000
Supplement	0
Page range	37-46
Number of pages	10
User name	Adonis
Cost centre	
PCC	\$21.50
Date and time	Friday, September 12, 2003 11:21:37 PM

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TARGETABLE GENE DELIVERY VECTORS

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1. CANCER THERAPY: TARGETING

The Holy Grail of cancer therapy, and a host of many therapies for major diseases, is to elicit a desired and selective biological effect on a specific cell type(s). In essence, cancer therapy is targeting. The key aspect of this therapy is in achieving a high rate of killing of cancer cells vs. normal cells. Accomplishing this has been extremely difficult for many reasons including the wide array of cell types involved, the systemic dissemination of cancer cells due to metastases, and the narrow biological differences between normal and cancer cells. While tremendous progress has been made in targeting, much still needs to be done, as current cancer therapies are clearly inadequate.

1.1. Conventional Therapy

In the past surgeons have tried to physically remove the tumor surgically without harming normal tissue. Even complete removal of a primary tumor does not ensure survival since earlier metastases to unknown sites in the body are left undetected. There is also some research, which even suggests that surgical intervention may enhance the growth of distant metastases due to removal of tumor cells producing angiogenesis inhibitors (O'Reilly *et al.*, 1994). Finally, in many cases the tumor grows back at the original site after surgical removal. Radiation aims to selectively destroy the most rapidly proliferating cells at the expense of the others. However, tumor cells can escape radiation therapy either by becoming resistant or by being in a non-dividing state during treatment. In addition, radiation is not always selective in that many normal cells are actively dividing and killed by the treatment. (Gastrointestinal cells, hair follicles, etc). Like radiation, chemotherapy is not completely selective and thus destroys many normal cells, and does not kill all tumor cells due to drug resistance and/or division state of the cell.

1.2. Immunotherapy

Immunotherapy is essentially the induction of the immune system to selectively expand a subset of cells and/or antibodies, which can target tumor cells. A variety of

approaches are currently in preclinical and clinical studies to attempt to boost the number and specificity of T cells, which will track and destroy tumor cells (For review see Rosenberg, 1998).

One approach, the use of monoclonal antibodies specific for tumor cells, was thought to be the great promise for cancer therapy approximately 20 years ago. However, most antibodies were generated in mice and elicited immune responses in humans, which effectively eliminated the therapeutic antibodies. However, they are now again showing great promise, as methods have been developed to humanize these antibodies. Of significant note are the clinical studies utilizing a humanized form of a monoclonal antibody (Herceptin) developed at Genetech that targets the HER2/neu receptors which are over-expressed on approximately 30% of breast cancer patients. Phase II trials have demonstrated a 65% increase in delay of disease progression in those patients receiving Herceptin and standard therapy compared with standard therapy alone (Robertson, 1998). This therapy again relies on the fact that systemically injected antibody binds selectively to tumor cells, and kills tumor cells via either inclusion of a toxin moiety fused to the antibody or in some cases the antibody may elicit a toxic effect through binding alone. Newer and more potent antibody-toxin fusions are becoming available, some of which can even extend the killing effect to surrounding tumor cells (Niculescu-Duvas, 1996 and references therein).

1.3. Barriers to Targeting

Tumors have an intricate and unusual blood supply network which renders binding of a molecule to the underlying tumor cell and support cells difficult. Tumors have high interstitial pressure, uneven and changing blood flow throughout the tumor, and an endothelial basement membrane barrier, all of which make the binding of any molecule to a significant fraction of tumor cells difficult (for review see Jain, 1997). In addition, tumors are composed of a multitude of cell types, which contribute to the overall ability of a tumor to thrive. Even the tumor cells within one patient can vary in genetic makeup and biological characteristics. Finally, any therapy must have the appropriate pharmacological profile *in vivo*, taking into account many parameters such as distribution and half-life.

2. CANCER GENE THERAPY: VECTORS

Gene therapy is essentially any method, which can deliver a desired gene(s) to a desired site(s), i.e. tumors, most often delivered to the site by gene therapy vectors. A vector is the substance, which carries the DNA to the desired state (nucleus of target cells) and can be synthetic, and/or viral based.

In vivo gene therapy for cancer for the most part has not involved inclusion of cell targeting but instead has focused on intra-tumoral or *in situ* injection of the vector. Some methods have been able to partially target the gene therapy vector by injecting into partially confined sites harboring metastases. These include the peritoneum (Deshane *et al.*, 1997), pleural space (Serman, 1998), and metastases in the liver (Anderson *et al.*, 1998). However, even treatment for partially localized tumor may not be efficient since metastases will spread and localize to many various organs throughout the body. Thus vector development must focus on systemically deliverable vectors which can achieve selective and reasonable transduction of tumor and/or tumor blood vessels.

Of course many of the barriers to chemotherapy and monoclonal antibody therapy will also hold true for viral or synthetic vectors. The vector must not be inactivated by the immune system or any other facet of the therapy and should be inert with the exception of binding to the desired cell type(s) within the tumor. Ideally the vector should remain in circulation only long enough to selectively transduce cells within any tumor mass. Once selective transduction is achieved then a multitude of cytotoxics and expandable therapies exist which should be able to be incorporated into vectors for effective therapy. These include HSV-Tk, cytosine deaminase, carboxypeptidase, p53 or other complementing genes, E1a, restriction of vector replication approaches, and the induction of immune responses (for review see Cusack *et al.*, 1998).

3. GENE DELIVERY VECTORS-PAST AND PRESENT ACHIEVEMENTS

Targeted gene delivery has been the emphasis in many gene delivery systems including retroviral, adenoviral, and synthetic vectors. Specific gene delivery to desired tissues or cell types can be "targeted" on multiple levels including transcriptional control through regulatable promoters and manipulation of the receptor specificity of the viral or non viral system. Recently, interest has focused on evaluating approaches for viral vector targeting by modifying the viral attachment protein in order to redirect the receptor specificity of the vector particles.

3.1. Adenoviral Vectors

Recently, work has focused on the development of targeted adenoviral vectors. Adenoviral vectors are useful for both *in vivo* and *in situ* gene delivery testing in animal tumor models and appear to be promising for potential therapeutic applications in humans including cancer and cardiovascular gene therapy. However, caution must be utilized in comparing efficiencies of vector targeting and efficacy in mice since mice are not the natural hosts of human adenoviruses. Adenoviral vectors when delivered systemically by intravenous administration in mice show a marked preferential transduction of liver cells although detectable levels of vector DNA can be found in most other tissues examined (Smith *et al.*, 1993). For reasons of safety and efficacy it will be important to control the cellular specificity of gene delivery by adenoviral vectors. One way to direct a vector to a specific target tissue or cell type is by selection of the route of administration (e.g. intratumoral, intranasal, or intramuscular delivery). In addition to the physical localization of gene delivery vectors, the transduction efficiency of these localized target cells may be increased by the addition of specific ligands and this may increase vector potency. Several approaches have been described to target adenoviral vectors and gene expression and are described below.

3.1.1. Transcriptional Regulation for Targeted Gene Expression. The use of regulatable or tissue specific promoters represents an additional strategy which will be of value in restricting the expression of the foreign gene to a desired cell type of tissue, particularly for cancer (for review see Clary *et al.*, 1998). A multitude of vectors have now been described utilizing tumor specific promoters to express cytotoxic genes for cancer therapy, including those from our own laboratory (Kaneko *et al.*, 1995). This general approach is

widely applicable as specific promoters could be incorporated into all currently used gene delivery systems and new promoters are rapidly being identified.

3.1.2. Targeting Adenoviral Vectors Via Fiber Modifications. While both the delivery route and tissue specific promoters will contribute to the cellular specificity which will be required for safe and effective therapies, a key factor in determining susceptibility of a particular cell to viral infection is the expression of receptors which permit viral attachment and entry. The capacity of human adenoviruses to bind to and infect a broad range of cultured cell lines and primary tissues from different species indicates that the adenovirus type 5, group C receptor is widely distributed and evolutionarily conserved. The identity of the group B coxsackievirus and group C adenovirus receptor (CAR) in human and mouse provides evidence for this observation (Bergelson *et al.*, 1997; Tomko *et al.*, 1997). One approach to selective cell transduction is to manipulate the adenovirus capsid in such a way as to redirect or change the receptor specificity and target the vector to a specific cell type or tissue. Targeting can be achieved by constructing an altered viral capsid which is no longer capable of binding to the normal cellular receptor, CAR but has acquired the ability to bind a specific cell type via a new receptor which has a defined and restricted pattern of expression. Selective transduction of tumor cells for example would be achieved by constructing a vector which selectively binds to a receptor which is overexpressed on the surface of tumor cells.

Attachment of the adenovirus particle to the cell is mediated by a high affinity interaction between the fiber protein and CAR (Wickham *et al.*, 1993). Following binding, virion cellular entry is mediated by an interaction between RGD peptide sequences in the penton base and cell surface integrins, which act as coreceptors (Wickham *et al.*, 1993). As a first step in the cellular transduction process, the interaction between the fiber protein and the cell is a logical target for controlling the cell specificity for transduction by adenoviral vectors. The other major adenoviral capsid proteins including hexon (Roy *et al.*, 1997) and penton (Wickham *et al.*, 1995), in addition to fiber (Karnasykh *et al.*, 1996; Stevenson *et al.*, 1997; Krasnykh *et al.*, 1997), have been modified to incorporate specific antigenic epitopes or receptor ligands.

The fiber protein is responsible for attachment of the virion to CAR cell surface receptors and various strategies have been attempted to incorporate novel receptor ligands into the fiber protein structure while still allowing for trimerization of the protein and subsequent capsid association. It was initially shown that the receptor specificity of an adenoviral vector could be changed from the Ad5 CAR receptor to the Ad3 serotype receptor by switching of the fiber head domains (Stevenson *et al.*, 1995; 1997). Also, adding a series of positively charged, lysine amino acid residues onto the C-terminus of the fiber protein allowed for the specific interaction with cell surface heparin sulfated proteoglycans and infection of cells which did not express CAR on the cell surface (Wickham *et al.*, 1996). Modified fiber proteins with novel receptor specificities can also be constructed by replacement of the fiber head domain with other trimeric proteins including head replacements from other naturally occurring adenovirus serotypes that utilize different cellular receptors (Stevenson *et al.*, 1995; 1997), fusion of peptide sequences to the fiber protein C-terminus (Michael *et al.*, 1995; Wickham *et al.*, 1996), or additions of peptide ligands within exposed loop regions of the fiber head domain (Xia *et al.*, 1994; Krasnykh *et al.*, 1998).

Successful targeting will depend on the identification and use of ligands with sufficient binding characteristics. Depending on the target cell type and the ligand selected, it may be necessary to ablate the native receptor tropism of the adenovirus fiber protein.

The crystal structure of the fiber knob domain has been reported (Xia *et al.*, 1994). Subsequent structure: function studies of the adenovirus fiber protein have demonstrated that many mutations will destroy the homotrimeric structure of the functional fiber which is required for assembly onto the viral capsid (Hong and Engler, 1996; Novelli and Boulanger, 1991). Recent studies have demonstrated that mutations of the fiber knob domain can be created that reduce fiber binding to CAR while still maintaining the ability to trimerize. A series of 6 individual amino acid substitutions in the fiber knob, DE loop between residues 460 to 471 reduced receptor binding by 100 fold (Robert Gerard, personal communication). Another approach to ablate the receptor-binding domain is to replace the fiber knob domain with a smaller peptide sequence that will drive trimerization of the fiber protein (Krasnykh *et al.*, 1998). Subsequent incorporation of these receptor binding negative fiber mutants into adenoviral capsids is still relatively new and untested but is a promising concept.

Multi-component systems have also been used to redirect the receptor tropism of adenoviral vectors. Bifunctional conjugates were constructed which consisted of a blocking anti-adenoviral fiber knob Fab linked to basic fibroblast growth factor 2 (Goldman *et al.*, 1997) or to folate (Douglas *et al.*, 1996). These adenoviral complexes successfully transduced cells that overexpressed either the FGF or folate receptor and which were previously refractory to adenovirus transduction. These systems while demonstrating effective retargeting and proof of concept have the disadvantage of not being amenable to large-scale production in preparation for human clinical trials.

Trans-complementation of a fiber-deleted adenoviral vector is an alternative approach to incorporate fiber proteins with novel receptor specificities into adenoviral vectors (Von Seggern *et al.*, 1998). This approach has several potential advantages compared to genetic modification of the adenovirus genome, including ease of switching fibers by preparing multiple cell lines that express novel fiber proteins and the ability to rapidly construct and incorporate multiple targeting ligands into adenoviral vectors. Preliminary studies utilizing this Ad5 fiber expressing cell line, 211B demonstrate successful incorporation of modified fibers into virion capsids. Both fiber genetic modification and trans-complementation may lead to the development of customized adenovirus vectors that selectively target specific cell types.

3.2. Targeted Retroviral Vectors

Modifications of the retroviral receptor ligand have been carried out to incorporate novel receptor binding domains to target specific cell types. The envelope protein of retroviral vectors has been modified to include various targeting ligands including ones for the human epidermal growth factor receptor (Han *et al.*, 1995), erythropoietin receptor (Kasahara *et al.*, 1994) and single chain antibody fragments against the LDL receptor (Somia *et al.*, 1995). Hall *et al.* (1997) have developed retroviral vectors which target and increase the transduction efficiency of vascular lesions through the incorporation of a high-affinity collagen-binding domain derived from von Willebrand clotting factor. There are many examples of incorporation of specific targeting ligands into retroviral vectors, however, due to the complex biology of the retroviral envelope protein, each modification of the retroviral envelope must preserve the additional functions associated with the envelope protein which produces infectious retroviral particles. Unlike adenovirus, where the fiber protein functions in only the initial attachment of the virion to cell surface receptors, the retroviral envelope gp70 protein is involved in the virion cell fusion process that must be maintained for successful targeted gene transfer.

3.3. Non-Viral Vector Gene Delivery

When administered systemically, non-viral vector complexes naturally target certain tissues including lung and tumor beds. Local administration of non-viral complexes has yielded the greatest success so far and many studies have been performed in tumor bearing animals (Yang *et al.*, 1998). Taking the field of drug delivery into account, many approaches for the use of targeting ligands for non-viral systems have been utilized and from these studies there are two major issues to be addressed for targeting (Woodle and Storm, 1998). First, there is a need to reduce "non-specific" interactions of the vector with unwanted biological cells and tissues. This then provides a foundation for addition of ligands for the desired specificity. Interestingly, just establishing vectors with reduced "non-specific" interactions can yield vectors that can accumulate in tumors, sites of infection, or sites of inflammation. The addition of ligands to such systems still has value in some cases but in and of itself can have sufficient added value to improved therapeutic responses. Sosnowski *et al.* (1996) for example, using FGF has demonstrated efficient ligand-mediated targeting of DNA to proliferating cells which overexpress the FGF receptor.

4. GENE DELIVERY VECTORS: FUTURE PROMISES AND CHALLENGES

4.1. Tumor Endothelial Cells as A Target

As has been previously discussed, there are several barriers to entry into tumors even when using high affinity ligands. For a variety of reasons, angiogenic endothelial cells may be the best choice for targeting and subsequent killing. Endothelial cells within tumors differ from normal quiescent vascular endothelial cells in many aspects including a dramatically increased proliferation rate, overexpression of a variety of proteins such as $\alpha_v\beta_3$ integrins and these cells are directly assessable to systemically delivered "targetable" vectors. In addition, the elimination of tumor endothelial cells is likely to amplify anti-tumor therapy since tumors cannot grow beyond 1–2 mm³ without a blood supply (for review see Folkman, 1996). In addition to targeting the vector to the angiogenic endothelial cells, several groups have reported promising therapy by expressing anti-angiogenic proteins systemically (for review see Folkman, 1998). Adenoviral vectors have been shown to systemically express therapeutic proteins into the circulation for at least one year in mice (Connelly *et al.*, 1998 and references therein). The adenoviral-mediated gene expression of anti-angiogenic proteins may result in sustained levels of therapeutic anti-angiogenic proteins that may selectively kill proliferating tumor cells systemically.

4.2. Expandable Vector Systems to Increase Therapeutic Efficacy

There are now a host of vector systems, in which the payload can be effectively amplified following the initial transduction of the tumor cell and/or endothelial cell. Chimeric vectors have recently been developed which can effectively increase the number of cells transduced with a vector via the utilization of an adenoviral vector which is capable of expressing retroviral genomes (Feng *et al.*, 1997). Tumor specific replication restricted vectors (TSRRV) are rapidly increasing in utility and use since they amplify

both the number of cells transduced and the amount of therapeutic gene product at the desired site. Replication restriction adenoviral vectors have been developed by our laboratory (Hallenbeck *et al.*, 1997) and others (Rodriguez *et al.*, 1997) by expressing a gene necessary for viral replication under the control of a tumor specific promoter. Another type of TSRRV involves the ability of particular cancer cells to complement specific gene defects in adenovirus (Bischoff *et al.*, 1996) or Herpes (Yazaki *et al.*, 1995). Clinical trials are currently ongoing which utilize the E1B deleted adenoviral vector (ONYX015) which has been shown to replicate in and selectively kill tumor cells which lack a functional copy of p53 *in vitro* and *in vivo* (Bischoff *et al.*, 1996). Another strategy involves utilizing an adenoviral vector, which expresses high affinity receptors at the tumor site. Following expression of the receptor, tumor cells are selectively eliminated utilizing radioactively labeled antibodies specific for the receptor (Raben *et al.*, 1996). There could be combinations of enzyme/prodrugs which again expand the therapeutic index of one vector. Finally, the use of tissue specific promoters and gene regulatory systems will permit high level but controlled gene expression. All approaches for the payload will be combined in various ways and are likely to be very effective once systemic and selective delivery of the vector to the tumor site is achieved.

5. IMMUNE EVASION

One of the most daunting challenges for the use of systemically delivered vectors is the avoidance of immune-mediated neutralization of the vector. Delivery of Ad5 based adenoviral vectors may especially be problematic since at least 50% of normal humans have neutralizing antibody. Nevertheless, there have been reports that suggest that these problems may be overcome by changing capsid components through altering immunogenic regions of capsid proteins (Roy *et al.*, 1998), by immunosuppression (Smith *et al.*, 1996), and/or by partially localized delivery. In addition, a recent report (Molnar-Kimber *et al.*, 1998) indicates that despite the existence of significant and preexisting humoral and cellular immunity to Ad5 in patients with mesothelioma, gene transfer of HSV-tk was not prevented when an adenoviral vector expressing HSV-tk was delivered intrapleurally.

6. SUMMARY

Adenoviral vectors, which have targeting ligands for tumor cells on the capsid, no natural tropism, and carry a therapeutic payload should be constructed soon and tested in pre-clinical models. Nevertheless, there are still important considerations for the design and therapeutic use of targetable vectors. Perhaps the single greatest challenge in the future, as it was in the past, will be finding ligands that have a higher apparent affinity for tumor and/or tumor endothelial cells than normal cells. However, the advent of many rapidly advancing technologies and information including the sequencing of the human genome, *in vivo* and *in vitro* phage display, rapid analysis of gene and protein expression in any context, and new cellular targets such as angiogenic endothelial cells, may provide many opportunities for the discovery of novel and useful ligands. In addition, the interests in targeting vectors are rapidly growing with new journals and meetings solely devoted to this subject increasing annually. Within the next 5 years, we should have meaningful clinical data on targetable vectors to reassess our progress.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Suzanne Forry-Schaudies and Sheila Connelly for critical review of the manuscript and Dr. Martin Woodle for advice and input.

REFERENCES

- Anderson, S.C., Johnson, D.E., Harris, M.P., Engler, H., Hancock, W., Huang, W.M., Wills, K.N., Gregory, R.J., Sutjipto, S., Wen, S.F., Lofgren, S., Shepard, H.M., and Maneval, D.C., 1998. P53 gene therapy in a rat model of hepatocellular carcinoma: intra-arterial delivery of a recombinant adenovirus. *Clin. Cancer Res.* 7:1649-1659.
- Bergelson, J.M., Cunningham, J.A., Droguett, G., Kurt-Jones, E.A., Krithivas, A., Hong, J.S., Horwitz, M.S., Crowell, R.L., and Finberg, R.W. 1997. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* 275:1320-1323.
- Bischoff J.R., Kirn D.H., Williams A., Heise C., Horn S., Muna M., Ng L., Nye J.A., Sampson-Johannes A., Fattaey A., and McCormick F., 1996. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science*. 274:373-376.
- Clary, B.M., and Lyerly, H.K., 1998. Transcriptional targeting for cancer gene therapy. *Surg. Oncol. Clin. N. Am.* 7:565-574.
- Connelly, S., Andrews, J.L., Gallo, A.M., Kayda, D.B., Qian, J., Hoyer, L., Kadan, M.J., Gorziglia, M.I., Trapnell, B.C., McClelland, A., and Kaleko, M., 1998. Sustained phenotypic correction of murine hemophilia A by in vivo gene therapy. *Blood*. 91:3273-3281.
- Cusack, Jr., J.C., and Tanabe, K.K., 1998. Cancer gene therapy. *Surg. Oncol. Clin. N. Am.* 7:421-469.
- Deshane, J., Siegal, G.P., Wang, M., Wright, M., Bucy, R.P., Alvarez, R.D., and Curiel, D.T., 1997. Transductional efficiency and safety of an intraperitoneally delivered adenovirus encoding an anti-erbB-2 intracellular single-chain antibody for ovarian cancer gene therapy. *Gynecol. Oncol.* 64:378-385.
- Douglas J.T., Rogers B.E., Rosenfeld M.E., Michael S.I., Feng M., and Curiel D.T. 1996. Targeted gene delivery by tropism-modified adenoviral vectors. *Nat. Biotechnol.* 14:1574-1578.
- Feng, M., Jackson, W.H., Goldman, C.K., Rancourt, C., Wang, M., Dusing, S.K., Siegal, G., and Curiel, D.T., 1997. Stable in vivo gene transduction via a novel adenoviral/retroviral chimeric vector. *Nat. Biotechnol.* 15:866-870.
- Folkman, J., 1996. Fighting cancer by attacking its blood supply. *Sci. Am.* 275:150-154.
- Folkman, J., 1998. Antiangiogenic gene therapy. *Proc. Natl. Acad. Sci. U.S.A.*, 95:9064-9066.
- Goldman C.K., Rogers B.E., Douglas J.T., Sosnowski B.A., Werbin Y., Siegal G.P., Baird A., Campain J.A., and Curiel D., 1997. Targeted gene delivery to Kaposi's sarcoma cells via the fibroblast growth receptor. *Cancer Research* 57:1447-1451.
- Hall F.L., Gordon E.M., Wu L., Zhu N.L., Skotzko M.J., Starnes V.A., and Anderson W.F., 1997. Targeting retroviral vectors to vascular lesions by genetic engineering of the MoMLV gp70 envelope protein. *Hum. Gene Ther.* 8:2183-2192.
- Hallenbeck, P.L., Chang, Y.-N., Hay, C., Golightly, D., Stewart, D., McGarrity, G., and Chiang, Y., 1996. Novel tumor specific replication competent adenoviral vector for gene therapy of cancer. *Abstracts of the fifth International Conference on gene Therapy of Cancer, November, 1998.*
- Han, X., Kasahara N., and Kan, Y.W., 1995. Ligand-directed retroviral targeting of human breast cancer cells. *Proc. Natl. Acad. Sci. USA.* 92:9747-9751.
- Hong J.S., and Engler J.A., 1996. Domains required for assembly of adenovirus type 2 fiber trimers. *J. Virology* 70:7071-7078.
- Kaneko S., Hallenbeck P., Kotani T., Nakabayashi H., McGarrity G., Tamaoki T., Anderson W.F., and Chiang Y.L., 1995. Adenovirus-mediated gene therapy of hepatocellular carcinoma using cancer-specific gene expression. *Cancer Res.* 55:5283-5287.
- Kasahara, N., Dozy, A.M., and Kan, Y.W., 1994. Tissue specific targeting of retroviral vectors through ligand-receptor interactions. *Science* 266:1373-1376.
- Krasnykh, V.N., Mikheeva, G.V., Douglas, J.T., and Curiel, D.T., 1996. Generation of recombinant adenovirus vectors with modified fibers for altering viral tropism. *J. Virology* 70:6839-6846.
- Krasnykh, V.N., Dmitriev, I., Mikheeva, G., Miller, C.R., Belousova, N., and Curiel, D.T., 1998. Characterization of an adenovirus vector containing a heterologous peptide epitope in the HI loop of the fiber knob. *J. Virology* 72:1844-1852.

- Krasnykh, V.N., Douglas, J.T., and Curiel, D.T., 1998. Tropism-modified adenoviral vectors for cell-specific gene delivery. *Abstracts of Cold Spring harbor Symposium "Gene Therapy", September, 1998.*
- Jain, R.K., 1997. The Eugene M. Landis award lecture 1996. Delivery of molecular and cellular medicine to solid tumors. *Microcirculation* 4:1-23.
- Michael, S.I., Hong, J.S., Curiel, D.T., and Engler, J.A., 1995. Addition of a short peptide ligand to the adenovirus fiber protein. *Gene Therapy* 2:660-668.
- Molnar-Kimber K.L., Sterman D.H., Chang M., Kang E.H., Elbash M., Lanuti M., Elshami A., Gelfand K., Wilson J.M., Kaiser L.R., and Albelda S.M., 1998. Impact of preexisting and induced humoral and cellular immune responses in an adenovirus-based gene therapy phase I clinical trial for localized mesothelioma. *Human Gene Therapy* 9:2121-2133.
- Niculescu-Duvaz, I., and Springer, C.J., 1996. Development of prodrugs for antibody-directed enzyme prodrug therapy (ADEPT). *Exp. Opin. Invest. Drugs* 5:289-308.
- Novelli A., and Boulanger P.A., 1991. Deletion analysis of functional domains in baculovirus-expressed adenovirus type 2 fiber. *Virology* 185:365-376.
- O'Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R.A., Moses, M., Lane, W.S., Cao, Y., Sage, E.H., and Folkman, J., 1994. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 79:315-328.
- Rabin, D., Buchsbaum, D.J., Khazaeli, M.B., Rosenfeld, M.E., Gillespie, G.Y., Grizzle, W.E., Liu, T., and Curiel, D.T., 1996. Enhancement of radiolabeled antibody binding and tumor localization through adenoviral transduction of the human carcinoembryonic antigen gene. *Gene Ther.* 3:567-580.
- Robertson, D., 1998. Genetech's anticancer Mab expected by November. *Nature Biotechnology* 16:615.
- Rodriguez, R., Schuur, E.R., Lim, H.Y., Henderson, G.A., Simons, J.W., and Henderson, D.R., 1997. Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Research* 57:2559-2563.
- Rosenberg, S.A., 1998. New opportunities for the development of cancer immunotherapies. *Cancer J. Sci. Am.* 4 Suppl. 1:S1-4.
- Roy, S., Shirley, P.S., McClelland, A., and Kaleko, M., 1998. Circumvention of immunity to the adenovirus major coat protein hexon. *J. Virol.* 72:6875-6879.
- Smith T.A., White, B.D., Gardner, J.M., Kaleko, M., and McClelland, A., 1996. Transient immunosuppression permits successful repetitive intravenous administration of an adenovirus vector. *Gene Ther.* 3:496-502.
- Somia, N.V., Zoppe, M., and Verma, I.M., 1995. Generation of targeted retroviral vectors by using single-chain variable fragment: An approach to in vivo gene delivery. *Proc. Natl. Acad. Sci. USA.* 92:7570-7574.
- Smith, T.A.G., Mehaffey, M.G., Kayda, D.B., Saunders, J.M., Yei, S., Trapnell, B.C., McClelland, A., and Kaleko, M., 1993. Adenovirus mediated expression of therapeutic plasma levels of human factor IX in mice. *Nat. Genet.* 5:397-402.
- Sosnowski B.A., Gonzalez A.M., Chandler L.A., Buechler Y.J., Pierce G.F., and Baird A., 1996. Targeting DNA to cells with basic fibroblast growth factor (FGF2). *J. Biol. Chem.* 271:33647-33653.
- Sterman D.H., Kaiser, L.R., and Albelda, S.M., 1998. Gene therapy for malignant pleural mesothelioma. *Hematol. Oncol. Clin. North. Am.* 12:553-568.
- Stevenson, S.C., Rollence, M.L., White, B., Weaver, L., and McClelland, A., 1995. Human adenovirus serotypes 3 and 5 bind to two different cellular receptor via the fiber head domain. *J. Virology* 69:2850-2857.
- Stevenson, S.C., Rollence, M.L., Marshall-Neff, J., and McClelland, A., 1997. Selective targeting of human cells by a chimeric adenovirus vector containing a modified fiber protein. *J. Virology* 71:4782-4790.
- Tomki R.P., Xu R., and Philipson L., 1997. HCAR and MCAR: The human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. *Proc. Natl. Acad. Sci. U.S.A.*, 94:3352-3356.
- Von Seggern, D., Kehler J., Endo R.I., and Nemerow G.R., 1998. Complementation of a fibre mutant adenovirus by packaging cell lines stably expressing the adenovirus type 5 fibre protein. *J. General Virology.* 79:1461-1468.
- Wickham, T.J., Mathias, P., Cheresh, D.A., and Nemerow, G.R., 1993. Integrins avb3 and avb5 promote adenovirus internalization but not virus attachment. *Cell* 73:309-319.
- Wickham, T.J., Carrion, M.E., and Kovesdi, I., 1995. Targeting of adenovirus penton base to new receptors through replacement of its RGD motif with other receptor-specific motifs. *Gene Therapy* 2:750-756.
- Wickham, T.J., Roelvink, P.W., Brough, D.E., and Kovesdi, I., 1996. Adenovirus targeted to heparin-containing receptors increase its gene delivery efficiency to multiple cell types. *Nature Biotechnol.* 14:1570-1573.
- Wickham, T.J., Segal, D.M., Roelvink, P.W., Carrion, M.E., Lizonova, A., Lee, G.M., and Kovesdi, I., 1996. Targeted adenovirus gene transfer to endothelial and smooth muscle cells by bispecific antibodies. *J. Virology.* 70:6831-6838.

- Woodle, M.C., and Storm, G. eds., 1998. *Long circulating liposomes, old drugs, new therapeutics*. Springer-Verlag Berlin-Heidelberg and Landes Bioscience, Georgetown, Texas.
- Yang, J., 1999. Direct intratumoral injection of naked DNA, in *Gene Therapy of cancer: methods and protocols*, (W. Walther and U. Stein, eds.), in press.
- Yazaki, T., Manz, H.J., Rabkin, S.D., and Martuza, R.L., Treatment of human malignant meningiomas by G207, a replication-competent multimutated herpes simplex virus 1. *Cancer Res.* 55:4752-4756.
- Xia D., Henry L.J., Gerard R.D., and Deisenhofer J., 1994. Crystal structure of the receptor binding domain of adenovirus type 5 fiber protein at 1.7 Å resolution. *Structure.* 2:1259-1270.